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TITLE: Investigating the Role of Radiation Therapy Breast Cancer Clinical and Translational Research

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CONTRACTING ORGANIZATION: The University of Pennsylvania
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13. ABSTRACT (Maximum 200 Words)

The training grant goals are to provide a broad range of opportunities for undergraduate students to participate in general clinical and basic science breast cancer research under the mentorship of experienced physician scientists in an academic institution. In the second year of the training grant, from May to August of 2004, six undergraduate students participated in original clinical or basic science research projects in the Department of Radiation Oncology. The students met or exceeded their expected goals of learning the principles of research, completing data collection and proceeding to analysis of the results. All students presented their research results at a min-symposium for the departmental staff and faculty. One student will be presenting his project as a poster presentation at the Era of Hope Conference in June 2005. One student who conducted basic science research is co-author on a published peer-reviewed paper, and the second student decided to continue in the same lab another year as a research fellow. Two students are a co-author on a manuscript in progress for submission to per-reviewed journals. One project is ongoing. The quality of applicants was high and their performance on their research projects was excellent.

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Breast cancer, radiation therapy

15. NUMBER OF PAGES
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Annual Summary Report
April 15, 2004- April 14, 2005

DAMD17-03-1-0309
Undergraduate Summer Training Program in Investigating the Role of Radiation Therapy Breast Cancer Clinical and Translational Research
Principal investigator: Eleanor E. R. Harris, MD

I. Introduction

This is the second annual summary report for the training grant, summarizing the first two years of the grant and primarily the accomplishments since the last annual report, for the summer 2004 time period. The training grant goals are to provide a broad range of opportunities for undergraduate students to participate in general clinical and basic science breast cancer research under the mentorship of experienced physician scientists in an academic institution. The students were exposed to the research process from design to analysis to authorship, with the goal of instilling both an understanding of the research process and of fostering a lasting commitment to the pursuit of breast cancer research. In the summer of 2004, from June through August, six undergraduates from the University of Pennsylvania and Haverford College participated in the research program. Each conducted an original research clinical or laboratory project under the supervision of a mentor. The majority of the students completed projects that have been or will be published. In their evaluations, the students expressed a high degree of satisfaction with their experience. As principal investigator, I was highly impressed with the quality of work and dedication these students exhibited. All six students exceeded expectations for the program's goals.

II. Body

The goal of the training grant is to recruit six students per summer for the duration of the grant period, and to provide each the opportunity to participate in a research project from start to finish. The initial phase is recruitment, which began in January 2004. Through contacts at the University of Pennsylvania School of Arts and Sciences, I was able to recruit an excellent pool of applicants from which to choose the six trainees. Recruitment flyers (attached) were submitted to the chairman of the Biological Sciences department, to the undergraduate premedical advisors office and to the Center for Undergraduate Research and Fellowships, the office which coordinates all research opportunities for the school. I received 30 inquiries and 15 complete applications for the six positions. The number of applicants was slightly lower this year than last year, likely because the posting of the notice on the website for the Center for Undergraduate Research and Fellowships was inadvertently delayed by their office. However, the caliber of applicants remained highly academic and diverse. Among the applicants, ten were women and five were men. Most of the applicants would be entering their sophomore or senior year, although two upcoming juniors also applied. Prior laboratory experience and science course were required for any applicants wishing to conduct basic science research, but no specific background was required for students wishing to pursue clinical research. I

selected six applicants from the pool and two alternates, and six students were accepted into the program. These included were four women and two men, four upcoming seniors, and two upcoming sophomores. Four students were assigned clinical research projects, and two were assigned laboratory projects.

Once the applicants were selected, they reviewed a project list giving the titles of possible research projects (attached). Prior to the beginning of the summer session, they were asked to review the list and select any projects titles they were interested in working on. I met with each candidate individually to discuss in greater detail the study questions and design involved in any of the projects they had found interesting. Students then submitted three choices for projects, and I assigned each one a specific topic. The students were then shown how to perform a Medline search and given copies of reference chapters from textbooks to read. Each was asked to independently research their specific topic by finding relevant background studies in the medical literature. I reviewed with them their background searches and provided them with any additional references they needed in order to learn about their topic. Once the students began their research in May 2004, they knew what their specific topic would be and had reviewed the relevant medical literature. During the summer session, all students were expected to spend at least one day shadowing a physician in the clinic to provide them a glimpse of the clinical nature of breast cancer patients' experiences.

The summer session was opened with an orientation session (agenda attached). This included an orientation to the hospital and Radiation Oncology Department, regarding payroll procedures, hospital and departmental policies, HIPAA training, departmental and hospital database access and utilization procedures, and the students were taken for hospital identification badges. The orientation included a series of lectures given by members of the Cancer Center faculty on breast cancer, radiation therapy and systemic therapy for breast cancer, clinical and laboratory research practices and documentation, clinical trials, and biostatistics. The trainees also heard presentations from residents and medical students who had done research work in the department as an example of the types of projects they would themselves be working on. The students then began their individual projects under the direction of their individual mentors. Three mentors participated in the program: Eleanor Harris MD, Lawrence Solin MD, and Gary Kao MD PhD; each mentor was assigned two students.

For clinical research projects, the first stage of work was to develop a study hypothesis and study design under the guidance of the faculty mentor. This step was completed in the first week of the project. For each clinical project, the students were provided with the pertinent patient list and an Excel spreadsheet containing numerous data points derived from the department's extensive breast cancer database. These data files were saved to a password protected departmental server and each student was assigned a unique username and password through which to access their specific data files and on which to save their work. Students were assigned desk with a computer workstation at which to work. Students then developed a list of data points they needed for their study and edited their spreadsheets. Students were also given the opportunity, with guidance,

to write and submit standard requests for Institutional Review Board approvals, which in all cases involved a simple expedited review process.

The second stage of work was data collection. Each student searched through the relevant medical records for the needed data and entered that data on their spreadsheets on an ongoing basis. During this phase, the students needed to think about their hypothesis and ensure that the data being obtained was adequate to analyze the study question. Each student kept a diary of their daily activities and met once a week with their mentor to discuss their progress and ask any questions that had arisen. I met with my own mentees as well as all four students weekly as well in order to assess the progress of each project. Either the faculty mentor or I met once weekly with the two students in the lab, to review their weekly logs and assess their progress. These students were under daily supervision of their faculty mentor, as they worked side by side with him in the lab.

The third stage of work was data analysis. For this, most of the students worked with a statistician. The students discussed with the statistician how to organize their data files for statistical analysis, then submitted their data files to her for computation. Simple analyses like descriptive statistics were sometimes performed by the student. Each student began work on an abstract in standard format (Introduction; Methods; Results; Conclusions).

The final stage of work was abstract production and presentation of their work. A research symposium was organized and held within the department at which each student gave a 20 minute Powerpoint presentation of their research project (attached). Each gave an introduction, followed by methods, results and conclusions. Although not a requirement, one student was second author on a manuscript by the completion of the summer project, and is now in press in Cancer Biology & Therapy.

At the end of the summer session, each student turned in their diaries and were asked to complete an evaluation form (attached) of the program. Their comments were generally positive, rating the quality of the introduction and orientation, mentoring and oversight, scope and interest level of the projects, experiences shadowing a clinician, interaction with other students and residents and facilities all in the excellent or good category. Some students expressed some frustration at obtaining their study charts for data collection in a timely manner. We have addressed this issue by relocating all of the study charts to a central location at the hospital and thus will not rely on delivery from a storage facility any longer. Many students felt that the research experience had solidified their interest in a medical or research career. Comments included: "I was previously turned away from laboratory research but I had a wonderful time and am really interested in the research we performed"; "The program increased my interest in Radiation Oncology as a field of medicine to pursue, specifically in regards to breast cancer research and treatments:; and "After this program I am considering getting my MD PhD".

All but one of the student's projects either has been presented or published, or is anticipated to be published in 2005.

III. Key Research Accomplishments:

Undergraduate researchers, 2004:

1. Jessica Liao: "Late Cardiac Effects of Breast Irradiation: An Analysis of Electrocardiograms"
Mentors: Eleanor Harris, MD and Candace Correa (medical student)
Status: Jessica completed data collection and preliminary analysis, She is co-author on a manuscript currently under revision, entitled "Late cardiac effects in left versus right sided early stage breast cancer patients treated with contemporary breast conservation therapy", to be submitted for peer-review publication by summer 2005. I anticipate several additional manuscripts from this data set, including one specifically analyzing the EKG findings that Jessica collated.

Jessica Liao initially completed this clinical research project in the summer training program; during the course of the program she was exposed to laboratory research, which kindled such interest that she performed Independent Study of mechanisms in cancer cells with Dr. Kao as part of her academic course load. This too has been successful and will likely result in a first-author manuscript to be submitted soon for consideration of publication.

2. Chandresh Ladva: "The Survival of Patients with Past Histories of Malignancies Prior to Early Stage Breast Cancer".
Mentor: Eleanor Harris, MD
Status: Chandresh completed all data collection and statistical analysis. His project has been accepted for poster presentation at the Era of Hope Conference in Philadelphia in June 2005. Chandresh wrote a draft manuscript, and the data is currently being updated with a revised manuscript in progress.

3. Rahn Voong: "Death and Degradation: Discovering the Molecular Determinants of Taxol Sensitivity"
Mentor: Gary Kao, MD, PhD
Status: Rahn is co-author on the following manuscript: Dowling M, Voong RK, Keutmann MK, Harris EE, Kao GD, "Mitotic Spindle Checkpoint Inactivation by Trichostatin A Defines a Mechanism for Increasing Cancer Cell Killing by Microtubule-Disrupting Agents". Cancer Biology & Therapy, Vol. 4 (2), In press, May 2005.

Rahn's research contributions were considerable and enabled not only her co-authorship on this project but also several others that have been submitted or are nearing completion including one in which she will be first-author. Rahn's experiences kindled such a strong desire to pursue cancer research, that she will devote a year following graduation to continue her work investigating mechanisms relating to breast and other forms of cancer.

4. Kristin Meliambro: "An Analysis of Outcomes of Distant Metastases in Unusual Histologies of Breast Cancer"
Mentor: Lawrence Solin, MD

Status: Kristin completed the majority of data collection and preliminary analysis. Final data collection is underway and manuscript preparation is to begin this year.

5. Andrea Denunzio: "Investigation of Cosmesis and Complications in Patients with DCIS"
Mentors: Lawrence Solin, MD and Neha Vapiwala, MD (resident)
Status: Andrea completed data collection and analysis. She will be co-author on a manuscript being written by a resident, Neha Vapiwala, with anticipated submission this year for peer-review publication.

6. Anil Maggee: "Density Determines Rapid Killing of Breast Cancer Cells by Taxol"
Mentor: Gary Kao, MD, PhD

Anil Maggee, the first student from outside Penn, solidified his desire to pursue a career as a physician. However, Anil was not able to complete a sufficient body of work for publication.

Update on Undergraduate Projects from 2003:

1. Jill Starzyk: "Analysis of biopsies performed after definitive irradiation for early stage breast cancer".
Mentor: Lawrence Solin, MD
Status: Accepted for poster presentation at the American Society for Clinical Oncology (ASCO) meeting in June 2004. Manuscript in preparation by a current resident.

Jill is currently a second year medical student at Northwestern University.

2. Eric A. Lee: "Outcomes after breast conservation therapy relative to Her2 expression" and "Factors that determine breast cancer cell resistance to the microtubule-disrupting drugs".
Mentors: Eleanor Harris, MD and Gary Kao, MD, PhD
Status: Clinical project accepted for oral presentation at the American Society for Therapeutic Radiology and Oncology (ASTRO) meeting in October 2004; "The Impact of Her2/neu Status on Local Recurrence in Women With Stage I-II Breast Cancer Treated With Breast Conservation Therapy", presented by Eleanor Harris, MD

First author on published manuscript: Lee EA, Keutmann MK, Dowling M, Harris EE, Chan G, Kao GD. "Mitotic Checkpoint Targets Human Cancer Cells to Killing by Microtubule- disrupting Drugs". *Molecular Cancer Therapeutics*,. Vol. 3(6): 661-669, 2004.

Co-author on manuscript submitted to the International Journal of Radiation Oncology, Biology and Physics in May 2005 entitled: "The Impact of Her2/neu Status on Local Recurrence in Women With Stage I-II Breast Cancer Treated With Breast Conservation Therapy", Authors: Eleanor E. R. Harris, MD, Wei-Ting Hwang, PhD, Eric A. Lee, James L. Rembert, MD, Michael D. Feldman, MD, PhD, Angela DeMichele, MD, Gary Kao, MD PhD, and Lawrence J. Solin, MD

Eric is currently a first year medical student at Duke University.

3. Michael Keutmann: "Inactivation of the Mitotic Checkpoint Targets Human Cancer Cells to Killing by Microtubule-disrupting Drugs"
Mentor: Gary Kao, MD, PhD
Status: Co-author on published manuscript: Lee EA, Keutmann MK, Dowling M, Harris EE, Chan G, Kao GD. "Mitotic Checkpoint Targets Human Cancer Cells to Killing by Microtubule- disrupting Drugs". *Molecular Cancer Therapeutics*,. Vol. 3(6): 661-669, 2004.

Co-author on published manuscript: Dowling M, Voong RK, Keutmann MK, Harris EE, Kao GD, "Mitotic Spindle Checkpoint Inactivation by Trichostatin A Defines a Mechanism for Increasing Cancer Cell Killing by Microtubule-Disrupting Agents". Cancer Biology & Therapy, Vol. 4 (2), In press, May 2005.

Michael is completing his senior year at the University of Pennsylvania.

4. Jordan Booty: "Time course of lymphedema development in breast cancer patients".
Mentors: Eleanor Harris, MD and Andrea Cheville, MD
Status: After Jordan completed his phase of the project, we decided to add some additional data points to the analysis. Data collection was continued in 2004 by a medical student, Neha Amin, who was supported by a grant from the NIH-funded Short Term Training Grants. She presented the updated work at the University of Pennsylvania School of Medicine Short Term Summer Research Student Symposium in August 2004. Statistical analysis is under way.

IV. Reportable Outcomes:

A. Publications or Meeting Presentations:

1. Dowling M, **Voong RK**, Keutmann MK, Harris EE, Kao GD, "Mitotic Spindle Checkpoint Inactivation by Trichostatin A Defines a Mechanism for Increasing Cancer Cell Killing by Microtubule-Disrupting Agents". Cancer Biology & Therapy, Vol. 4 (2), In press, May 2005.

2. **Ladva C,** Harris EE, Hwang W-T, Solin LJ, "The Survival of Patients with Past Histories of Malignancies Prior to Early Stage Breast Cancer". Accepted for poster presentation at the Era of Hope Conference, June 2005, Philadelphia, PA

3. Harris, EE. "Undergraduate Summer Training Program Investigating the Role of Radiation Therapy in Breast Cancer Clinical and Translational Research", Accepted for poster presentation at the Era of Hope Conference, June 2005, Philadelphia, PA.

B. Manuscripts in Progress:

1. Harris EE, Correa C, Hwang W-T, **Liao J,** Litt HI, Ferrari V, Solin LJ. "Late cardiac effects in left versus right sided early stage breast cancer patients treated with contemporary breast conservation therapy". Manuscript in progress.

2. Vapiwala, N, **Denunzio A**, Harris, EE, Hwang W-T, Solin LJ. "Investigation of Cosmesis and Complications in Patients with DCIS". Data analysis and manuscript preparation in progress.

V. Conclusions:

In the second year of the training grant, encompassing the summer of 2004, I was able to recruit an academically stellar group of student researchers of diverse interests and backgrounds, all of whom worked diligently and who in many cases exceeded the expectations for the training program goals. One is a co-author on an in-press peer-reviewed manuscript, one will be presenting his work at the 2005 Era of Hope Conference, and several manuscripts based on these students work are being analyzed or written for peer-review submission by me or residents in the department.

The students seemed highly satisfied with their experience and all stated on their evaluations that the program increased their interest in pursuing a career in medicine and biomedical research, and specifically their interest in working in the area of breast cancer research.

I have addressed the main area of concern for the students, which was easy access to their study charts, by bringing all the archived study charts into a single repository in our hospital offices. This year we have nearly doubled our requests for applications, and have another high caliber group of students poised to begin their summer projects.

VI. References:

1. Dowling M, **Voong RK**, **Keutmann MK**, Harris EE, Kao GD, "Mitotic Spindle Checkpoint Inactivation by Trichostatin A Defines a Mechanism for Increasing Cancer Cell Killing by Microtubule-Disrupting Agents". Cancer Biology & Therapy, Vol. 4 (2), In press, May 2005.

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4. **Lee EA**, **Keutmann MK**, Dowling M, Harris EE, Chan G, Kao GD. "Mitotic Checkpoint Targets Human Cancer Cells to Killing by Microtubule- disrupting Drugs". *Molecular Cancer Therapeutics*,. Vol. 3(6): 661-669, 2004.

5. Harris EE, **Starzyk J**, Solin LJ. "Results of biopsies performed after breast conservation therapy for stage I-II breast cancer", Proceedings ASCO, Vol. 23: 86, 2004.

6. Harris Eleanor E, Hwang Wei-Ting, **Lee Eric**, Cengel Keith, Angela DeMichele, Solin Lawrence J. "Her2/neu Status Does Not Impact Local Recurrence in Women With Stage I-II Breast Cancer Treated With Breast Conservation Therapy", *International Journal of Radiation Oncology,Biology and Physics*. Vol 60 (1) S: S135, 2004

VII. Appendix

1. Recruitment Flyer

2. Orientation Agenda

3. Evaluation Form

4. Symposium Schedule and Research Student Powerpoint Presentations

Undergraduate Breast Cancer Research Opportunity

Release: December 2004

The Department of Radiation Oncology at the University of Pennsylvania School of Medicine is pleased to announce a new research opportunity for undergraduates at the University of Pennsylvania to participate in investigative projects in the area of breast cancer research. The training program will provide a broad range of opportunities for students to participate in general clinical or basic science breast cancer research. The student will be exposed to the research process from design to analysis to authorship, with the goal of instilling both an understanding of the research process and of fostering a lasting commitment to the pursuit of breast cancer research.

Due to a generous grant from the Department of Defense Breast Cancer Research Program, we are able to offer a $4000 stipend to up to six undergraduates students able to spend 12 weeks participating in a full-time mentored research project. (For interested students, additional independent study during the academic year may be available.) The program director is Eleanor Harris, MD, assistant professor of Radiation Oncology. In order to apply for the training program, students must be in good academic standing at the university. Students are required to fill out an application form and to submit an unofficial transcript. Students must also submit a one-page essay describing their reasons for applying to the training program, in particular their motivation for conducting breast cancer research. Applications will be due no later than April 9, 2004.

Students requesting laboratory projects involving bench research will be required to have some prior experience in basic laboratory techniques involved in their experiments, as 12 weeks is too brief to allow training in techniques and the completion of a set of experiments. For students interested in clinical projects, no prior research experience will be required. In fact, it is anticipated that the training program will provide many of these students with their first exposure to high quality scientific research with expert faculty mentoring

Dr. Harris will review the applications and choose participants based on their interests, commitment, academic record, motivation and future goals. Applicants will be informed of their acceptance by mid-April 2004. Shortly afterwards, each participant will meet with Dr. Harris to discuss their background, areas of interest and potential research ideas. If the student has a specific research project or faculty member in mind, he or she will be put in contact with the appropriate mentor. While the primary mentor must be a training program faculty, students may be assigned a co-mentor from another department if beneficial. If the student does not have a specific research goal at the initial meeting, he or she will be given a list of projects with brief descriptions to review and will be asked to choose three projects from that list. Dr. Harris will review all the requests and assign a project to each participant. The student will then meet with that faculty mentor to discuss the specific project. The student will be assigned background reading, and with the mentor's guidance will be asked to develop a timeline for research plan. It is anticipated that participants will complete these steps during the spring semester and prior to beginning their project in the summer session.

To request an application, please contact Ms. Betsy Patton in the Department of Radiation Oncology by phoning 215-662-3094, or by writing to email address: patton@xrt.upenn.edu.

Orientation
Breast Cancer Summer Research Program

June 1, 2004
Plaza A Conference Room
3rd Floor Founders

09:00 - 10:00	Mark Patrick and Betsy Patton Laboratory Administrator and Program Coordinator
10:00 – 11:00	Susan Domchek, MD Medical Oncology
11:00 – 11:30	Peggy Alfarano HIPPA
11:30 – 12:00	Neha Vapiwala, MD Lecture
12:00 – 01:00	Lunch
01:00 – 05:00	Eleanor Harris, MD Radiation Oncology

June 2, 2004
Conference Room
Basement Founders

09:00 – 09:30	Linda Miller, BSN, RN Director of Nursing Clinical Research
09:30 – 10:30	Ryan Smith, MD Lecture
10:30 – 11:30	Ralph Ferro Computer Training
11:30 – 12:00	Candace Correa, Medical Student Lecture
12:00 – 01:00	Lunch
01:00 – 01:30	Betsy Patton Database Coding
01:30 – 03:00	Wei-Ting Hwang, PhD Biostatistics and Epidemiology
03:00 – 05:00	Gary Kao, MD, PhD Scientific Research Methods

Breast Cancer Research in Radiation Oncology – 2003 Summer Training Program
Evaluation Form

Name:_____ Date:_____

Faculty Mentor:_____

ProjectTitle:_____

_____

Part A: For the following aspects of the summer research training program, please choose the best option describing the quality of each part of the program, if applicable to your project:

	Excellent	Good	Satisfactory	Inadequate
1. Breast cancer introductory lecture series				
2. Departmental/Hospital orientation				
3. Research methods and design				
4. Clarity of research project				
5. Mentoring and oversight				
6. Access to mentor				
7. Scope of research project appropriate				
8. Shadowing a clinician				
9. Support staff/ file room access				
10. Laboratory facilities				
11. Supplies				
12. Office/computer facilities				
13. Interaction with residents/students				
14. Technical services				
15. Interaction with department personnel				

Part B: Please assess the following goals of the research training program, and whether they were accomplished during the program:

1. Did the program provide an opportunity to learn more about breast cancer research?

2. Was the project you were assigned or designed of the appropriate scope and level of difficulty?

3. Was the project of sufficient interest to you?

4. Did the program increase your interest in medical research?

5. Did the program increase your interest in breast cancer research?

6. Did the program change or affect your educational or intellectual goals? If so, how?

7. What do you think were the goals of your research program, and were they met?

8. Would you recommend this program to a peer?

Part C: Your comments and suggestions will be very helpful in improving the quality of the research training program. Please discuss any of your ideas for improvements or suggestions for changes.

Undergraduate Breast Cancer Summer Research Symposium
Friday, August 13, 2004

Investigation of Cosmesis and Complications in Patients with DCIS
Speaker: Andrea DeNunzio

The Survival of Patients with Past Histories of Malignancies Prior to Early Stage Breast Cancer
Speaker: Chandresh Ladva

Late Cardiac Effects of Breast Irradiation: An Analysis of Electrocardiograms
Speaker: Jessica Liao

Death and Degradation: Discovering the Molecular Determinants of Taxol Sensitivity
Speaker: K. Ranh Voong

Effects of Initial Nodal Status, Patterns of Distant Metastases, and Her2/neu Status on Outcome in Metastatic Breast Cancer
Speaker: Kristin Meliambro

Density Determines Rapid Killing of Breast Cancer Cells by Taxol
Speaker: Anil Magge

Investigation of Cosmesis and Complications in Patients with DCIS

Andrea DeNunzio

Co-Mentor: Lawrence J. Solin, MD
Co-Mentor: Neha Vapiwala, MD

Overview

- Retrospective Study Designed to:
  - Evaluate Factors Affecting Cosmesis
  - Determine Complications Associated with Treatment
  - Evaluate Factors Affecting Occurrence of Complications

Background

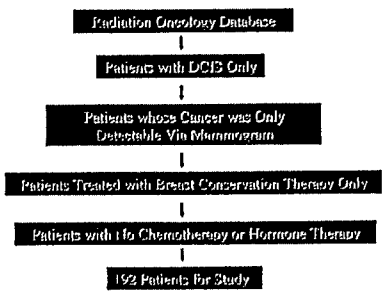
- DCIS Diagnosed in Symptomatic Patients
- Advent of Mammography
- Diagnosis of DCIS in Asymptomatic Patients
- 15-20% of All New Breast Cancers Are DCIS

Silverstein, Melvin J. *Annals of Surgical Oncology*. 6(8):802-803.

Background

- DCIS Is Manageable
- Comparable Cause-Specific Survival Rates for Mastectomy and Breast Conservation Therapy Treatments
- Focus On Other Aspects of Treatment to Improve Quality of Life

Methods: Population Selection



Methods: Selection of Potential Factors

- Previously Studied Factors:
  - Dose (Total, Whole Breast, and Tumor Bed Boost)
  - Fractionation
  - Volume of Excision
  - Breast and Cup Size
  - Weight
  - Age
  - Race
  - Axillary Dissection

Methods: Data Collection

Potential Factors:

- Age
- Race
- Body Mass Index (BMI)
- Bra Size and Cup Size
- Tangential Chest Wall Separation
- Volume of Resected Tissue
- Scar Length
- Number of Surgeries
- Dose Inhomogeneity
- Use of Wedges
- Axillary Dissection
- Sentinel Node Biopsy
- Presence of Moist Desquamation

Methods: Data Collection

- Record and Verify Previously Reported:
  - Cosmesis Scores Assigned During Follow-Up Examinations
  - Complications Experienced By Each Patient

Methods: Data Analysis

- Creation of Cosmesis Timeline:
  - Use Only Month and Year to Determine Time Interval
  - Designate Each Month as a Number (Ex. June = 6)
  - Subtract the Year and Month of Completion of Radiation Therapy from the Year and Month of Cosmetic Evaluation, respectively
  - Use Magnitude and Sign of the Month Difference to Fine-Tune the Year of Cosmetic Evaluation

Methods: Data Analysis

- Cosmesis:
  - Range: 1-16 Years
  - Median Time: 6 Years
  - Over 5 Years: n=73
  - Over 10 Years: n=21

Methods: Data Analysis

- Creation of Complications Timeline
  - Creation Similar to Cosmesis Timeline
  - Determination of Relevant Factors:
    - Whether or Not the Patient Experienced a Given Complication
    - Duration of Complication

Methods: Data Analysis

- Complications:
  - Breast Edema
  - Arm Edema
  - Decreased Range of Motion
  - Cellulitis

Future Analysis

- Finish Data Collection
  - Pathology Reports
  - Patient Films
- Finish Timelines
- Data Sort to Determine Relevant Links
- Meet With Statistician

Acknowledgements

Special Thanks To:

Dr. Harris
Betsy Patton

Dr. Vapiwala
Dr. Solin

P-Crew

In the beginning...

- Cancer plagued women...

and then it did again

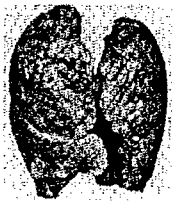
You had what now?:

The Survival of Patients with Past Histories of Malignancies Prior to Early Stage Breast Cancer

From the fingertips of
Chandresh Ladva

A look into prior malignancies

- Very little published
- French study with extrapulmonary malignancies prior to lung cancer
  - Massard G, et. al. (2000)



Methods

- Database screen
  - female
  - DCIS, Stage I, or II
  - prior malignancy
  - no contralateral breast cancer
- 63 patients
- Chart Review
- Matched
  - stage
  - age +\- 2 yrs.
  - date of diagnosis +\- 5yrs.
- Statistical analysis

Data collected

- Survival
- Family History
- Breast and Prior Malignancy Treatments

Statistical Analysis

- Continuous variables
  - age
  - total RT dosage
  - Students' t-test
- Categorical variables
  - family history
  - treatment methods
  - χ^2 test for independence
- Kaplan-Meier curves
  - survival
    - ahem...not quite done yet!

Patient Characteristics

+/- Family History:

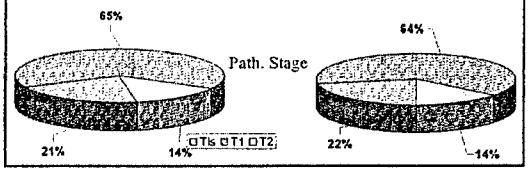
BC-- p= .8510; OC-- p= .0325

Both-- p= .2900

Supraclav-- p= 1.000

Post. Ax.-- p= .4028

IMN-- p= .0065

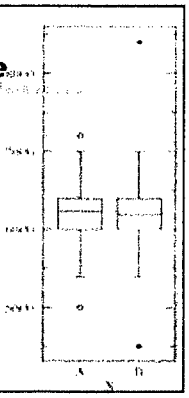


Pt. Char. - RT Dosage

- Comparison
  - A= Pts. w/ PMH of cancer
  - B= Pts. w/o PMH of cancer

Total RT Dosage:

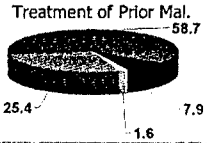
p= .8857



Cases with detail

Interval Between Cancers (Months)	
Mean	104.7
Median	62
Standard Deviation	106.3

Treatment of Prior Mal.



■ Surgery Only ■ Radiation Only
□ Chemotherapy Only ■ Combinations

Cases by Predominant PMs

Site of Prior Malignancy		Cervical		Colon		Skin/Basal Cell		p-Values
No			7		8		8	
		%	n=	%	n=	%	n=	
Mean Age			48 (29-60)		66 (49-79)		51 (28-66)	
Pathologic Stage								
	Tis	14.3	1	25	2	0	0	0.3399
	T1	57.1	4	62.5	5	100	8	0.1110
	T2	28.6	2	12.5	1	0	0	0.2605
	N0	85.7	6	75	6	87.5	7	0.7778
	N1	14.3	1	12.5	1	12.5	1	0.9682
	Unkown	0	0	12.5	1	0	0	0.3753
Median RT Dose to Tumor Bed (cGy)			6120		6050		6400	
Chemotherapy		28.6	2	25	2	62.5	5	0.2439
Hormones		42.9	3	37.5	3	37.5	3	0.9711

Survival

Survival Status

Status	+PM (n=)	-PM (n=)
Alive, NED	49	51
Alive with Disease	0	1
Dead, NED	0	0
Dead of Disease	9	8
Dead of Unknown Cause	5	3

Follow-up Time in Months

	+PM	-PM
Mean	80.95238095	85.84126984
Median	64	70
Standard Deviation	57.14876698	60.66837576

p= 0.5218

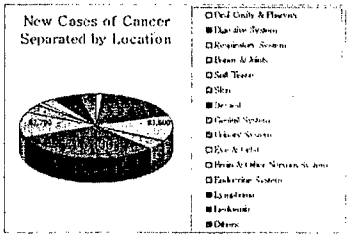
Conclusions

- Similarity shown between groups
  - Characteristics
  - Follow-up time
  - Survival status
- No difference in survival regardless of presence of previous malignancy
- Not confirmed

Late Cardiac Effects of Breast Irradiation: An Analysis of Electrocardiograms

Jessica Liao
Co-mentor: Dr. Eleanor Harris
Co-mentor: Candace Correa
Department of Radiation Oncology
Hospital of the University of Pennsylvania
8/13/04

Estimated New Cases of Cancer in Females in the US, 2003



Source: American Cancer Society, 2003

Why Study Cardiac Diseases?

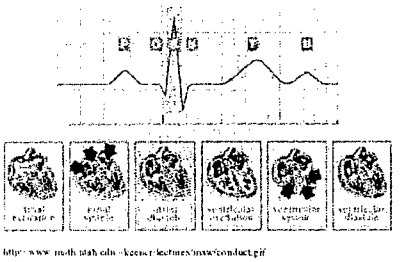
- Accidental heart irradiation
- Many methods to analyze cardiac diseases
- EKG is gold standard for sinus rhythm

Advantages and Disadvantages of using EKGs

☺Advantages:
- Easy to obtain
- Results not subjective

☹Disadvantages:
- Inaccurate identification of MIs

Electrical Currents cause the Waves and Complexes seen on an EKG



http://www.math.utah.edu/~keenan/electrines/anim/conduct.gif

Incidence of Heart Disease and Functional Significance of Changes in the Electrocardiogram 10 years After Radiotherapy for Breast Cancer. Strender, et. al. (1986)

- 197 patients evaluated for heart disease
- Conclusions:
  - Increase in cardiac abnormalities after radiation, specifically ST and T-wave changes
  - The incidence of serious cardiac complications is low

ST and T-Wave Changes are Possible Indicators of Many Cardiac Diseases

- Myocardial Ischemia
- Myocardial Infarction
- Ventricular Hypertrophy

Our Study vs. Strender's Study

- Chemotherapy Drugs
- Sample Size
- Before/After plus Left/Right

Research Method

- Screen Patients:
  - Had breast cancer radiation therapy
  - Has EKG history
- Collect Data:
  - EKG abnormalities
  - Chemotherapy
  - Radiation Doses and Fields
- Statistical Analysis:
  - χ^2 test
  - t-test assuming unequal variance

Patient Characteristics for All Patients

TABLE 1
PATIENT CHARACTERISTICS - ALL PATIENTS

VARIABLES	ALL LEFT-SIDED n =	%	ALL RIGHT-SIDED n =	%	p-Value
No. of patients	327	49.5%	333	50.5%	
Mean Age	54.5	-	54.4	-	0.90
Original Chemotherapy					
No	234	71.6%	217	65.2%	0.08
CMF	69	21.1%	82	24.6%	0.28
CAF	19	5.8%	28	8.4%	0.19
Tamoxifen	96	29.4%	112	33.6%	0.24
Other	5	1.5%	6	1.8%	0.74
Mean Radiation dose (in cGy)	6297	-	6212	-	0.24

Research Groups

Patient Characteristics for Patients with Pre-XRT EKGs

TABLE 2
PATIENT CHARACTERISTICS - PATIENTS WITH PRE-XRT EKGS

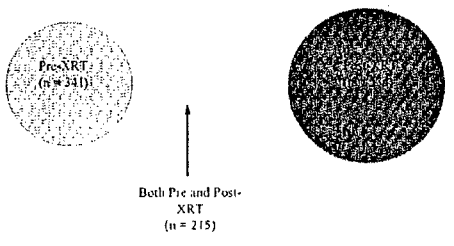
VARIABLES	LEFT-SIDED n =	%	RIGHT-SIDED n =	%	p-Value
No. of patients	166	48.7%	175	51.3%	
Mean Age	55.9	-	57.8	-	0.16
Original Chemotherapy					
No	115	69.3%	113	64.6%	0.36
CMF	36	21.7%	42	24.0%	0.61
CAF	10	6.0%	17	9.7%	0.21
Tamoxifen	66	39.8%	78	44.6%	0.37
Other	5	3.0%	3	1.7%	0.43
Mean Radiation dose (in cGy)	6311	-	6246	-	0.18
Supraclavicular	24	14.5%	37	21.1%	0.11
Posterior Axillary	6	3.6%	17	9.7%	0.02
Internal Mammary Nodes	10	6.0%	8	4.6%	0.55

Patient Characteristics for Patients with Post-XRT EKGs

TABLE 3
PATIENT CHARACTERISTICS - PATIENTS WITH POST-XRT EKGS

VARIABLES	LEFT-SIDED		RIGHT-SIDED		P-Value
	n =	%	n =	%	
No. of Patients	269	50.4%	265	49.6%	
Mean Age	53.7	-	54.5	-	0.43
Original Chemotherapy					
No	197	73.2%	172	64.9%	0.04
CMF	53	19.7%	66	24.9%	0.15
CAF	15	5.6%	23	8.7%	0.16
Tamoxifen	77	28.6%	82	30.9%	0.56
Other	4	1.5%	4	1.5%	0.95
Secondary Chemotherapy					
No	187	69.5%	188	70.9%	0.72
CMF	9	3.3%	3	1.1%	0.08
CAF	3	1.1%	3	1.1%	0.99
Tamoxifen	63	23.8%	54	20.4%	0.34
Other Cardiotoxics	5	1.9%	10	3.8%	0.18
Mean Radiation dose (in cGy)	6290	-	6291	-	0.95
Supraclavicular	56	20.8%	80	30.2%	0.01
Posterior Axillary	21	7.8%	33	12.5%	0.03
Internal Mammary Nodes	29	10.8%	18	6.8%	0.10

Research Groups



Patient Char. for Group Containing Both Pre and Post-XRT EKGs Validate Groups with Only Pre or Post-XRT EKGs

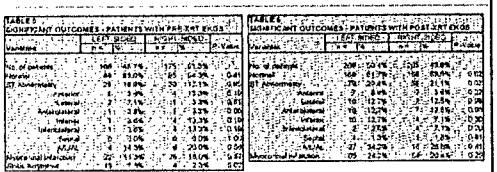
TABLE 4
PATIENT CHARACTERISTICS - PATIENTS WITH BOTH PRE-POST-XRT EKGS

VARIABLES	LEFT-SIDED		RIGHT-SIDED		P-Value
	n =	%	n =	%	
No. of patients	109	50.2%	107	49.8%	
Mean Age	57.0	-	56.8	-	0.90
Original Chemotherapy					
No	78	72.2%	68	63.6%	0.17
CMF	20	18.5%	26	24.3%	0.30
CAF	6	5.6%	12	11.2%	0.13
Tamoxifen	47	43.5%	48	44.9%	0.84
Other	4	3.7%	1	0.9%	0.18
Secondary Chemotherapy					
No	65	60.2%	65	60.7%	0.94
CMF	4	3.7%	1	0.9%	0.18
CAF	0	0.0%	0	0.0%	1.00
Tamoxifen	38	35.2%	31	29.0%	0.33
Other Cardiotoxics	0	0.0%	2	1.9%	0.16
Mean Radiation dose (in cGy)	6300	-	6283	-	0.72
Supraclavicular	17	15.7%	25	23.4%	0.16
Posterior Axillary	5	4.6%	9	8.4%	0.26
Internal Mammary Nodes	3	2.8%	5	4.7%	0.46

Significant Outcomes

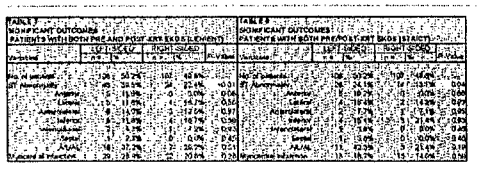
- 38 different cardiac diseases
  - Pre-xrt abnormalities – 54%
  - Post-xrt abnormalities – 61%
  - *P*-value <0.04

Significant Outcomes



- This retrospective study indicates that the incidence of ST abnormality is increased in left-sided breast cancer patients compared with right-sided breast cancer patients ($p = 0.03$).

Significant Outcomes for Patients with Both Pre and Post-XRT EKGs



- Compared with comparable right-sided patients, left-sided breast cancer patients experienced a statistically significant increase ($p<0.05$) in ST abnormalities after breast irradiation
- No difference in specific locations
- No difference in myocardial infarctions

Discussion

- The percentage of EKG abnormalities noted before and after radiation were much higher than previously reported by the Strender group.
- Results corroborate their findings of a higher incidence of ST abnormalities after radiation.
- Difference of cardiac diseases due to breast irradiation of L vs. R

Future Work

- Are similar results in the increased incidence of cardiac diseases shown using other tests?
- Could prescription drugs be affecting our results?
- Since the likelihood of developing ischemia is higher, is this radiation to the left breast actually decreasing their overall survival?

References

1. http://www.cancer.org/downloads/STT/CAFF2003PWSecured.pdf

2. Strender, L.E. et al. (1986) Incidence of Heart Disease and Functional Significance of Changes in the Electrocardiogram 10 years After Radiotherapy for Breast Cancer. *Cancer* 57:929-934.

3. Gustavsson A. et al. (1999) No Serious Late Cardiac Effects After Adjuvant Radiotherapy Following Mastectomy in Premenopausal Women with Early Breast Cancer. *Int. J. Radiation Oncology Biol. Phys.*, Vol. 43, No. 4: 745-754.

4. http://www.math.utah.edu/~ keener/lectures/maw/conduct.gif

Acknowledgements

- Eleanor Harris, MD
- Candace Correa
- Betsy Patton

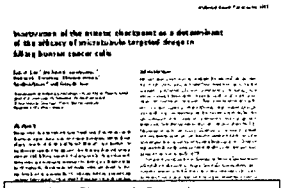
Death and Degradation: Discovering the Molecular Determinants of Taxol Sensitivity

K. Ranh Voong
Radiation Oncology Department
University of Pennsylvania
August 13, 2004

Accomplishments

- Discovered Preliminary Evidence that:
  - Taxol may cause the *accelerated degradation* of checkpoint proteins
  - Mechanism may be the *activation* of proteins involved in Programmed Cell Death
- Became skilled with diverse laboratory techniques
- Writing manuscript with Anil Magge

What are the Mitotic Checkpoint Proteins?



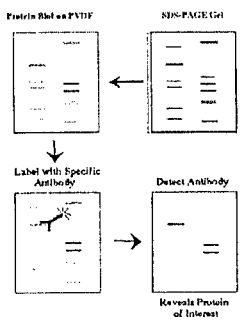
- "Mitotic Checkpoint" proteins (BubR1, CENP-E, Bub1, etc.) enforce Mitotic cell cycle block
- When inactivated or destroyed: leads to mitotic catastrophe

Methods

Protein Analysis Protocol

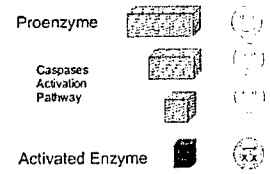
1. Plate out cells
2. Treat cells.
3. Harvest for pellets.
4. Lyse cells.
5. Run samples on SDS-PAGE gel.
6. Transfer protein to membrane.
7. Probe for proteins of interest.
8. Expose and Develop film

Western Blot Procedure



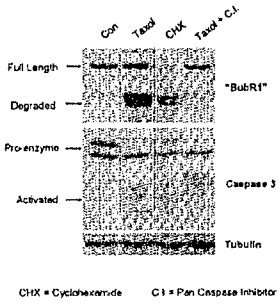
Taxol leads to degradation of Mitotic Checkpoint Proteins

What are Caspases?



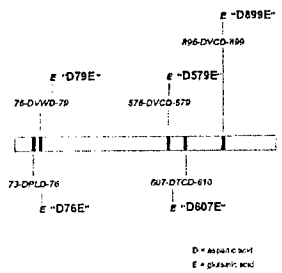
- Are a family of Proteases that are themselves activated by cleavage (Proenzyme > Activated)
- Cleave/activate other proteins, ultimately bringing about cell death.

Taxol Activates Caspase to degrade BubR1

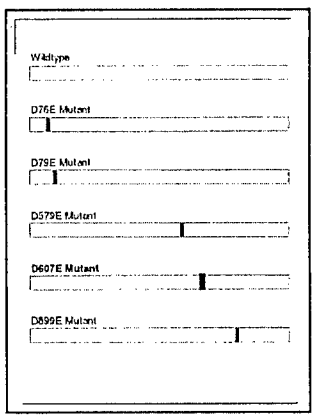


CHX = Cyclohexamide C.I = Pan Caspase Inhibitor

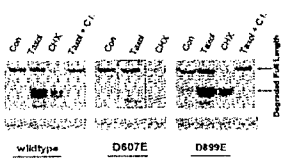
Targeted Mutations of Every Potential Caspase Cleavage Site in BubR1



- Caspases recognize "Dead Box" cleavage motifs DxxD



BubR1 Instability Decreased with Specific Mutation of Caspase Cleavage Site



Conclusions

- Key Mitotic Checkpoint proteins are actually Unstable
- Taxol leads to accelerated degradation of BubR1 by activating Caspases
- Aspartic Acid 607 ("D607E") defines the prime Caspase cleavage site in BubR1

Future Implications

- Functional consequence of expressing BubR1 mutants
  - Make cells more resistant to Taxol?
- Find specific Caspase involved in Taxol mediated killing
- Confirm that Caspases cleave other mitotic checkpoint Proteins

Acknowledgments

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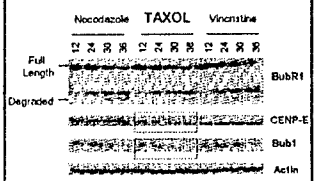
  - Dr. Mijin Kim
  - Wes Baff & Katie Murphy
  - Melissa Dowling
  - Dr. Fang Liu
  - Shary Parker
- Betsy Patton

Taxol Unmasks the Instability of Mitotic Checkpoint Proteins



Effects of Initial Nodal Status, Patterns of Distant Metastases, and Her2/neu Status on Outcome in Metastatic Breast Cancer:

The University of Pennsylvania Experience

Kristin Meliambro
August 13, 2004

Metastatic Breast Cancer

- Most common sites of metastasis, in order of frequency:
  - ***Bone***: ~ 25% of breast cancers spread first to bone
  - ***Lung***: ~60-70% of women who die from breast cancer had it spread to lungs
    - Only site of distant mets in 21% of cases
  - ***Liver:*** ~67% of women with metastatic breast cancer eventually have spread to liver
  - ***Less common:*** Brain, Bone Marrow, Ovaries, Eye, & Other areas

http://imaginis.com/breasthealth/metastatic.asp

Treatment for Distant Mets

- Majority of treatments focus on alleviating symptoms **(palliative treatment)**, improving quality of life, prolongation of life
  - ***Surgery***: i.e. isolated lungs mets, pathologic fractures
  - ***Radiation Therapy***: i.e. painful bony mets, unresectable CNS mets (brain, meningeal, spinal cord), following surgery

http://imaginis.com/breasthealth/metastatic.asp

Treatment for Distant Mets

- ***Systemic Therapies***:
  - *Chemotherapy*: i.e. CMF, CAF, AC
  - *Hormone Therapy*: if tumor is ER-positive, PR-positive, ER/PR unknown
    - i.e. Tamoxifen, Taxol, Aromasin, Herceptin (for Her-2/*neu* positive receptors on tumor cells)

http://imaginis.com/breasthealth/metastatic.asp

Research Methods

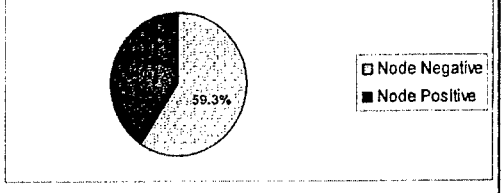
Patient Selection:

- **Separated out patients w/ distant mets**
- **Time frame: (start date XRT) 1977-2000, to allow for follow-up time**
- **Categorized by:**
  - **pathology of primary breast cancer**
  - **nodal status (node negative v. node positive)**
- **Excluded:**
  - **patients with prior malignancy**
  - **patients with no surgical evaluation of axilla- (nodal status unknown)**

Research Methods

- **Total Number of Patients: 204** (possibly 230)
  - **Intraductal/Infiltrating Ductal & Infiltrating Ductal: 181 patients**
    - 105 node negative; 76 node positive
  - **Infiltrating Lobular: 12 patients**
    - 8 node negative; 4 node positive
  - **Infiltrating Ductal/Lobular: 4 patients**
    - 1 node negative; 3 node positive
  - **Medullary: 3 patients**
    - 2 node negative; 1 node positive
  - **Colloid: 4 patients** (all node negative)
  - **Squamous Cell: 1 patient** (node negative)

Breakdown of Patients



Study Questions

- Looking for relationships between:
  - median survival time for distant mets and initial nodal status
  - number and types of metastatic sites and initial nodal status
  - median survival time and combination of metastatic sites (eg: bone/brain v. bone/lungs v. lung/liver, etc)

Research Methods

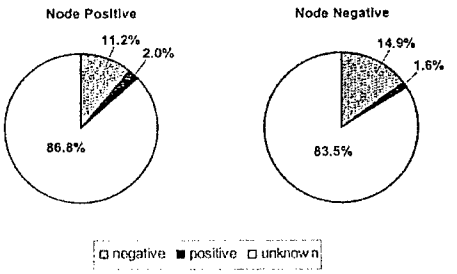
- Reviewed ~200 charts for following data per patient:
  - median survival time (months)
  - dates of each diagnosis of distant mets
  - number of distant mets sites at each diagnosis
  - total number of distant mets sites
  - chemotherapy per incident of distant mets
  - Her2/*neu* status

Her2/neu status & Distant Mets

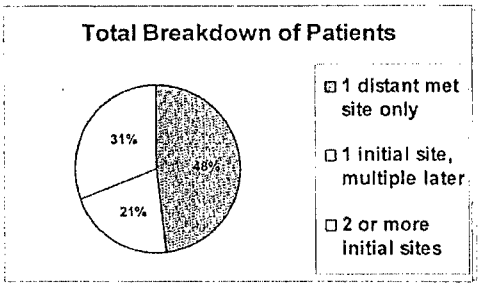
- HER2/*neu* is overexpressed in 20-30% of Br. Cas
  - tumor cells overexpressing HER2/*neu* may have up to 2 million copies of receptor on surface (compared to 20,000-50,000 copies in normal breast epithelial cells)
- HER2/*neu* overexpression correlates w/ more aggressive behavior, shortened disease free survival, and overall survival rates
  - marker of response to chemotherapy and HT
- **Question: Relationship between HER2/*neu* status and Distant Metastasis of Breast Cancer?**

http://www.emedicine.com/med/topic2808.htm

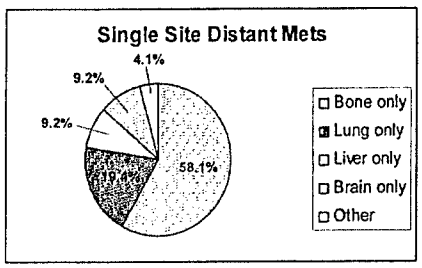
Her2/neu status



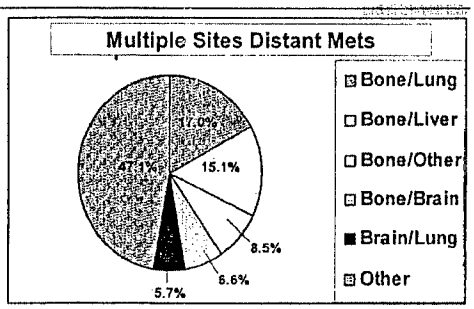
Preliminary Results



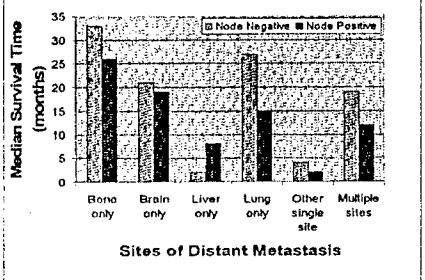
Preliminary Results



Preliminary Results



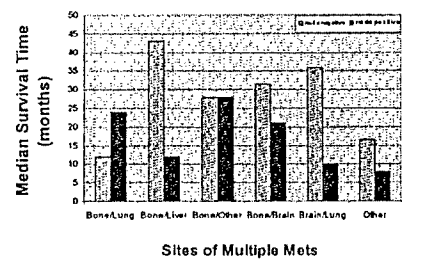
Median Survival Time v. Sites of Distant Mets



Observations

- Single site mets only:
  - longer median survival time for node negative patients except those with *liver mets*
  - greatest difference in median survival time between node negative and node positive patients with *lung mets* (12 months)
- Longer median survival time for node negative and node positive patients with distant mets to *bone only, brain only,* and *lung only* compared to node negative and node positive patients with multiple site mets

Median Survival Time v. Sites of Multiple Mets



Observations

- Longer median survival time for node negative patients for all multiple site mets combinations except:
  - *bone/brain* – equal median survival time
  - *bone/lung*- longer median survival time for node positive patients
- Greatest difference in median survival time between node negative and node positive patients with *bone/liver mets* (31 months), followed by *brain/lung mets* (26 months)

Future Study Questions

- Is there a difference in median survival time between node negative and node positive patients with:
  - single site distant mets?
  - multiple site distant mets?
- For patients with multiple distant mets, is outcome better or worse depending on sequence of diagnosis, i.e. simultaneous diagnosis of > 2 sites v. sequential diagnosis of > 2 sites?

Future Study Questions: Her2/neu

- Is there a difference in the rate of development of distant mets between Her2/*neu* positive & Her2/*neu* negative patients for:
  - single site distant mets?
  - multiple site distant mets?
- How does Her2/*neu* status affect median survival time?
- How might Her2/*neu* status affect future treatment recommendations?

Acknowledgements

Special Thanks to:
Dr. Harris
Dr. Vapiwala
Dr. Solin

Density Determines Rapid Killing of Breast Cancer Cells by Taxol

Anil Magge
August 13, 2004
Department of Radiation Oncology
University of Pennsylvania

Accomplishments

- Became skilled with diverse laboratory techniques
- Identified a novel factor – CELL DENSITY – that determines if cancer cells can be rapidly killed by Taxol
- Discovered that specific sequencing with Radiation Therapy may reverse the resistance of densely growing cells to Taxol
- Writing manuscript with Ranh Voong

Cell Density Project

Background

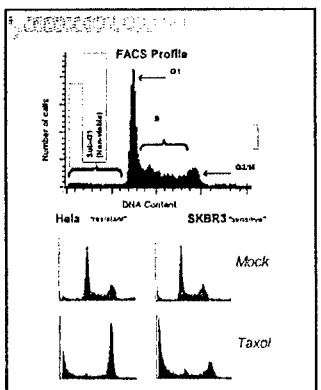
- microtubules = "skeleton" of cell
  - dynamic: constantly forming and disassembling
  - Forms mitotic spindle
- disrupt microtubule dynamics→ cell death
  - TAXOL prevents disassembly
  - Vincristine, Nocodazole prevent formation
- cell death mechanism unclear

Cell Density Project

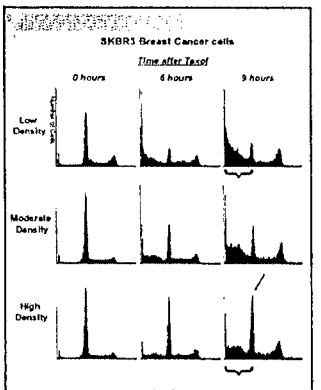
FACS and the Cell Cycle

- FACS (Flow Assisted Cytometric Sorting) measures the amount of cells in certain parts of the cell cycle by analyzing the DNA content

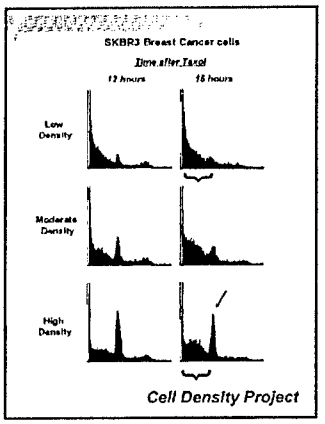
- G1- "Gap 1"→pause before Synthesis
- S-phase→cell replicates its DNA
- G2- "Gap 2"→ pause before Mitosis
- M-Mitosis→ separating of the chromosomes

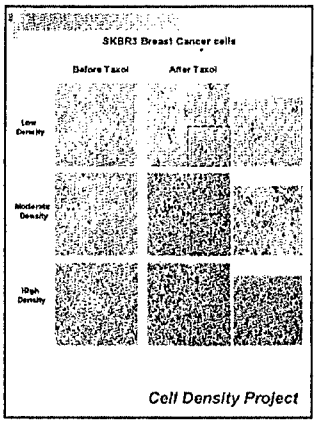


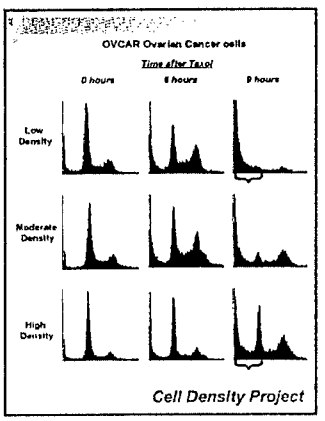
Cell Density Project

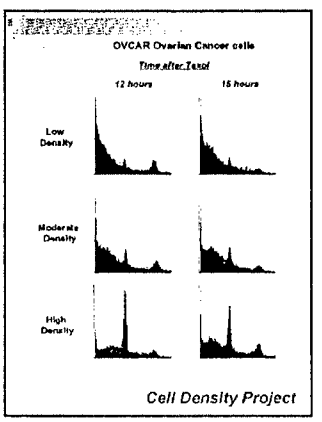


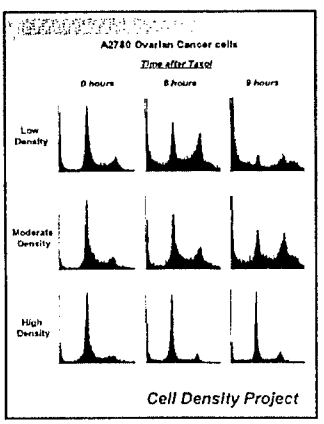
Cell Density Project

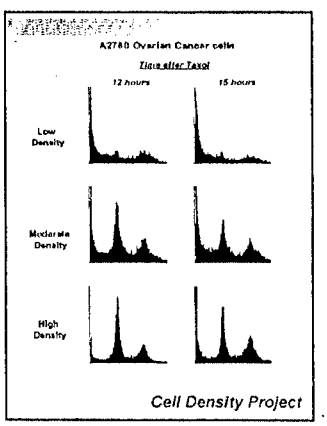

SKBR3 Breast Cancer cells
Time after Taxol
12 hours
16 hours
Low Density
Moderate Density
High Density
Cell Density Project

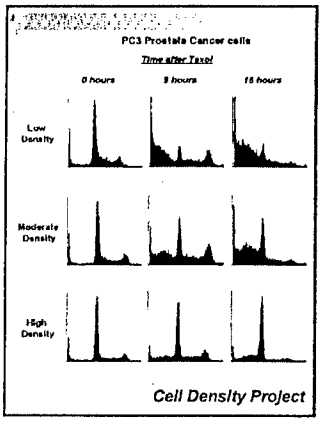

SKBR3 Breast Cancer cells
Before Taxol
After Taxol
Low Density
Moderate Density
High Density
Cell Density Project

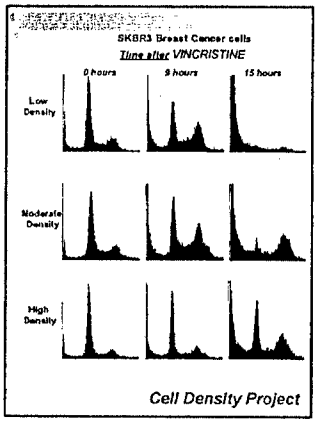

OVCAR Ovarian Cancer cells
Time after Taxol
0 hours
6 hours
9 hours
Low Density
Moderate Density
High Density
Cell Density Project

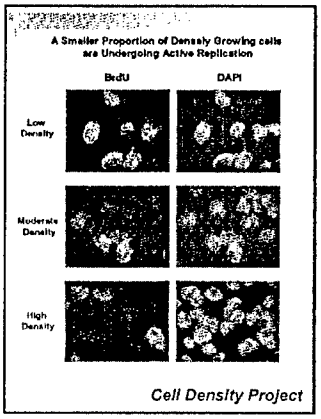

OVCAR Ovarian Cancer cells
Time after Taxol
12 hours
16 hours
Low Density
Moderate Density
High Density
Cell Density Project


A2780 Ovarian Cancer cells
Time after Taxol
0 hours
6 hours
9 hours
Low Density
Moderate Density
High Density
Cell Density Project


A2780 Ovarian Cancer cells
Time after Taxol
12 hours
16 hours
Low Density
Moderate Density
High Density
Cell Density Project



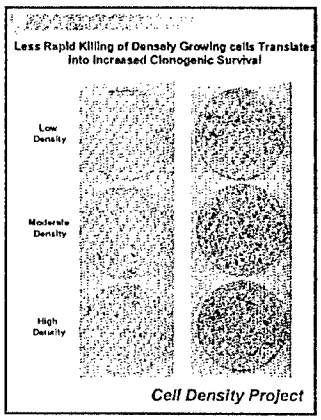




"What are the CLINICAL implications of our findings?"

Experimental Protocol: Clonogenic Assay

1. Grow cancer cells under Sparse, Moderately Dense, or Dense conditions
2. Treat all cells with Taxol for six hours
3. Harvested cells, counted, and re-plated identical cell numbers into fresh plates with Taxol-free media
4. Let grow undisturbed for 10 days.
5. Stain and count colonies



Conclusions

- Sparsely growing breast cancer cells are more easily killed by Taxol than Densely growing cells
- This in fact may be a general phenomena; also seen with OVCAR, A2780, PC3,...
- This may explain why LARGER tumors may respond poorer to chemotherapy

Cell Density Project

Conclusion

- Sparsely growing breast cancer cells are more easily killed by Taxol than Densely growing cells

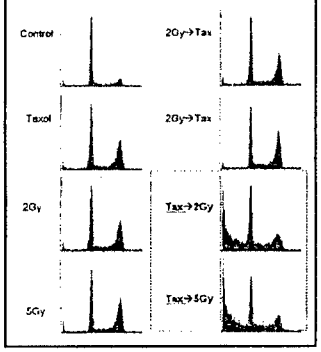
How can we sensitize Densely Growing cells to Taxol?

Cell Density Project

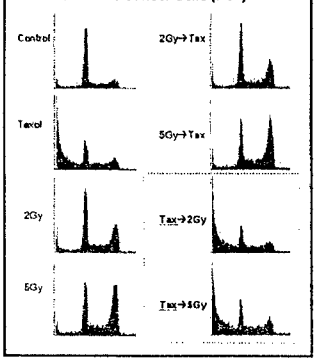
ANSWER:

- With specific order of cancer treatment combination
- **TAXOL BEFORE IR** pulse leads to more cell death than the converse

Taxol Before Radiation Kills More Breast Cancer Cells (SKBR3)



Taxol Before Radiation Kills More Prostate Cancer Cells (PC3)



Conclusions II

- Sparsely growing breast cancer cells are more easily killed by Taxol than Densely growing cells
- However the resistance of cells grown at high density can be partially reversed by treating with Taxol first then following it with Radiation

Cell Density Project

Acknowledgments

- **Dr. Eleanor Harris**
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  - Melissa Dowling
  - Dr. Mijin Kim
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  - Shary Parker
  - Dr. Fang Liu
- Betsy Patton

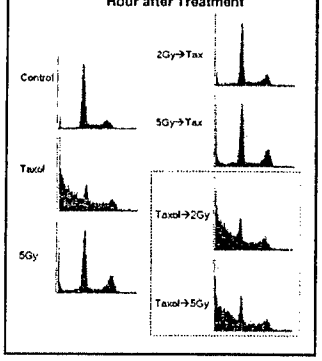
Future Directions

- Show these effects in animal models of breast cancer
- Explore combinations of different chemotherapies with Taxol

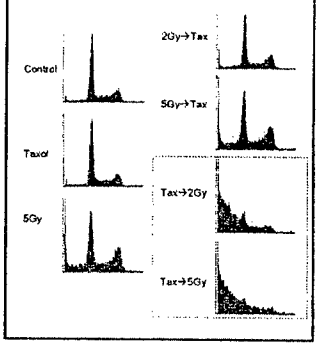
Paper Supporting Taxol before IR Treatment Sequence

Cell Cycle–Dependent Antagonistic Interactions between Paclitaxel and γ-Radiation in Combination Therapy

Taxol before IR Kill More SKBR3 Cells 24 Hour after Treatment



Taxol before IR Kill More PC3 Cells 24 Hour after Treatment



Methods and Materials

Protocol for Taxol-IR Sequence Experiment

1. Plate out cells densely
2. Treat with Taxol or radiation
3. Incubate for 12 hours. Wash cells. Treat with radiation or Taxol.
4. Incubate 12 hours. Harvest Cells.
6. Wash cells that will be harvested 12 hours later.
6. Harvest remaining samples
7. Process and run Flow Assisted Cytometry (FACS)

What about the future?

- Possible inquiries and improvements
  - Expanding study
    - Larger population
    - Longer follow-up
      - Simplicity allows easier long term data collection for patients in database
  - Comparison of cause of prior cancers and relationship with BC
    - Other cancers likelier to couple with BC
    - Genetic, hormonal, chemogenic, etc.

Hasta luego...

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.